

--Production of a F(ab')<sub>2</sub> version of the humanized anti-IL-8 6G4V11N35A Fab was accomplished by constructing a fusion protein with the yeast GCN4 leucine zipper. The expression plasmid p6G4V11N35A.F(ab')<sub>2</sub> was made by digesting the plasmid p6G425chim2.fab2 with the restriction enzymes bsaI and apaI to remove the DNA sequence encoding the 6G4.2.5 murine-human chimeric Fab and replacing it with a 2620bp bsaI-apal fragment from pPh6G4.V11N35A. The plasmid p6G425chim2.fab2 is a derivative of pS1130 which encodes a fusion protein (the GCN4 leucine zipper fused to the heavy chain of anti-CD18) and the light chain of anti-CD18 antibody. The expression plasmid p6G4V11N35A.F(ab')<sub>2</sub> was deposited on February 20, 1996 with the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209, U.S.A. (ATCC) and assigned ATCC Accession No. 97890. A pepsin cleavage site in the hinge region of the antibody facilitates the removal of the leucine zipper leaving the two immunoglobulin monomers joined by the cysteines that generate the interchain disulfide bonds. The DNA and protein sequence of the h6G4V11N35A.F(ab')<sub>2</sub> are depicted in Figs 35-37.--

Please replace the paragraph beginning at page 240, line 21, with the following rewritten paragraph:

--In the rabbit model of ear ischemia reperfusion injury, antibody was administered intravenously at a single dose (5 mg/kg) at the time of reperfusion. In this model, ischemia reperfusion injury is characterized by tissue damage, edema and sometimes necrosis; all attributable in part to neutrophil-mediated damage. Monitoring of ear volume over time is a surrogate end-point for evaluating edema in the ear tissue. The resulting data (depicted in Fig. 71) showed that treatment with 20 kD linear PEG-, 30 kD linear PEG- and 40 kD branched PEG-conjugated Fab's effectively reduced ear swelling and edema at all time points of observation (days 1, 3 and 5). In fact, the efficacy of all three PEGylated Fab's was statistically indistinguishable from that of the full length IgG murine anti-rabbit IL-8 monoclonal antibody 6G4.2.5 at all time points observed. These data support the efficacy of large effective size anti-IL-8 Fab'-PEG conjugates in ischemic reperfusion injury and specifically support the ability of 40 kD branched PEG-conjugated Fab' molecules to reach and act on disease effector targets in circulation and other tissues.